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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,245,224**, on August 14, 1998, by **THE UNIVERSITY OF BRITISH COLUMBIA**, assignee of Ian Clark-Lewis and Jiang-Hong Giong, for "Chemokine Receptor Antagonists and Chemotherapeutics".

PRIORITY DOCUMENT

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Date

Abstract

Peptides corresponding to the N-terminal 9 residues of stromal cell derived factor-1 (SDF-1) have SDF-1 activity. SDF-1, 1-8, 1-9, 1-9 dimer and 1-17 induced intracellular calcium and chemotaxis in T lymphocytes and CEM cells, and bound to CXC chemokine receptor 4 (CXCR4). The peptides had similar activities to SDF-1, but were less potent. Whereas native SDF-1 had half maximal chemoattractant activity at 5 nM, the 1-9 dimer required 500 nM and was therefore 100 fold less potent. The 1-17 and a 1-9 monomer analog were 4- and 36-fold, respectively less potent than the 1-9 dimer. Both the chemotactic and calcium response of the 1-9 dimer was inhibited by an antibody to CXCR4. The basis for the enhanced activity of the dimer form of SDF-1, 1-9 is uncertain but it could involve an additional fortuitous binding site on the 1-9 peptide in addition to the normal SDF-1, 1-9 site. A 1-9 analog, 1-9[P2G] dimer, was found to be a CXCR4 antagonist. Overall this study shows that the N-terminal peptides are CXCR4 agonists or antagonist and these could be leads for high affinity ligands.

Introduction.

SDF-1 is a member of the chemokine family of structurally related proteins with cell chemoattractant activity (1). Although many chemokines have pro-inflammatory roles, SDF-1 appears to have a fundamental role in the trafficking, export and homing of bone marrow cells (2, 3). It is produced constitutively, and particularly high levels are found in bone-marrow stromal cells (4, 5). A basic physiological role is implied by the high level of conservation of the SDF-1 sequence between species (4). In vitro SDF-1 stimulates chemotaxis of a wide range of cells including monocytes and bone marrow derived progenitor cells (2, 5). Particularly notable is its ability to stimulate a high percentage of resting and activated T lymphocytes (5, 6). It is the only known ligand for CXCR4, a 7 transmembrane receptor that has been variously described as LESTR (7), HUMSTR (8) and Fusin (9). CXCR4 is widely expressed on cells of hemopoietic origin, and is a major co-receptor for HIV-1 (9). Consistent with this dual role of CXCR4, SDF-1 blocks HIV-1 entry into $CD4^+$ cells (10, 11).

The SDF-1 sequence indicates that it belongs to the CXC family of chemokines, but has only about 22% identity with other chemokines (5). Despite the divergent primary structure, the recently described 3-dimensional structure indicates that it has a similar fold to other chemokines (12). Furthermore, structure-activity analysis of SDF-1 (12) indicated the importance of N-terminal residues 1-8 for binding, and of residues 1 and 2 for receptor activation. Residues 12-17 located in the loop region also contribute to binding. In the SDF-1 structure, the region N-terminal to the CXC motif is highly disordered, but the loop region immediately following the CXC motif is well defined at least in its backbone atoms. These two regions have been identified as being important in other CC and CXC chemokines (12-15). As with other chemokines, N-terminal modification of SDF-1 led to dissociation of binding and activity (12). Thus despite the difference in primary structure, from both a structural and a functional perspective, the general mechanism of receptor binding is similar for SDF-1 and other chemokines.

The key role of the N-terminal region of the SDF-1 protein in receptor binding and activation suggests that the N-terminal region alone could be sufficient for binding or activity. Here we show that peptides

corresponding to the N-terminal region bind CXCR4 and have SDF-1 activity. A dimer of SDF-1, 1-9 was the most potent of the peptides tested.

Experimental Procedures

Peptide Synthesis. The peptides were prepared as described previously (13). The peptides were purified by HPLC and analyzed by mass spectrometry. tBoc-α-aminobutyric acid was used to prepare the analog SDF-1, 1-9[Aba 9] which had CH_2-CH_3 in place of CH_2SH. The 1-9 and 1-17 peptides were dimerized via a disulfide bridge formed by gentle oxidation of the cysteines using 10% DMSO in water. Following HPLC purification dimer formation was verified, by mass spectrometry

Cell preparation and culture. Human peripheral blood mononuclear cells were isolated from donor blood buffy coats by centrifugation on Ficoll-Paque. The cells were treated with phytohemagglutinin (1.0 $\mu g.ml^{-1}$) and expanded in the presence of IL-2 (100 $U.ml^{-1}$) for 7 to 17 days as described (16). These cells are referred to as "T lymphocytes". CEM cells, a human lymphoblastoid $CD4^+$ T cell line (ATCC, Rockville MD), was cultured in RPMI medium containing 15 $\mu g.ml^{-1}$ of 8-azaguanine (Aldrich Chemical Company, Milwaukee WI) and 10% FCS.

Chemotaxis. Migration of T lymphocytes was assessed in 48 well chambers (NeuroProbe, Cabin John MD) using collagen-coated polyvinylpyrrolidone-free polycarbonate membranes with 3 μm pores (16). Migrated cells were counted in five randomly selected fields at 1000x magnification after migration of 1 h. Disposable Transwell trays (Costar, Cambridge MA) with 6.5 mm diameter chambers and membrane pore size of 3 μm, were used to assay chemotaxis of CEM cells. The agonist, in Hepes-buffered RPMI 1640 supplemented with 10 $mg.ml^{-1}$ BSA (0.6 ml), was added to the lower well, and 0.1 ml of CEM cells ($1 \times 10^7.ml^{-1}$) in the same medium without agonist was added to the upper wells. In some experiments the monoclonal antibody 12G5 (17, R&D Systems, Minneapolis MN)) was preincubated with the cells at 10 $\mu g.ml^{-1}$ for 15 min at 0 °C. The antibody was also added to the lower well at 10 $\mu g.ml^{-1}$. After 2 h, cells that migrated to the lower wells were counted. Chemotactic migration was determined by subtraction of cells migrated in medium alone. All assays were performed in duplicate.

SDF-1 Receptor Binding. Competition for binding of ^{125}I-labeled SDF-1 to CEM cells was carried out as described (12). MCP-1 and RANTES binding was measured on THP-1 cells as documented elsewhere (14).

$[Ca^{2+}]_i$ changes. T lymphocytes and CEM cells loaded with Fura-2 were stimulated with the indicated agonist, and the $[Ca^{2+}]_i$-related fluorescence changes were recorded from 0-60 s (18). Receptor desensitization was tested by monitoring changes during sequential additions at 60 s intervals. Where indicated the cells were preincubated with the 12G5 antibody prior to chemokine treatment.

Results

SDF-1 N-terminal peptides are chemotactic. The N-terminal region of SDF-1 is directly involved in receptor recognition and activation (12), and therefore we tested peptides corresponding to the N-terminal region for activity. The sequences of the peptides are shown in Figure 1. Both the 1-8 and 1-9 peptides induced dose-dependent chemotaxis of CEM cells (Fig. 2a). The concentrations required for 50% of the maximal response (EC50) are summarized in Table 1. The 1-9 peptide was about 1,000-fold less potent than native SDF-1. However the 1-9 was 7-fold more potent than the 1-8 peptide. The peptides were also tested on T lymphocytes (Fig. 2b) and the results were similar to those obtained with CEM cells, except that the T lymphocytes were less responsive to SDF-1 or the peptides. The results clearly show that the 1-9 and 1-8 peptides have SDF-1 like activity, but relatively low potency. The chemoattractant activity of 1-9 was fully inhibited by the SDF-1 antagonist, SDF-1, 1-67[P2G] (12), but not by an IL-8 antagonist which blocks CXCR1 (19) (Fig. 3a). These findings suggest that despite its lower potency the 1-9 peptide is similar in its mechanism of CXCR4 activation to native SDF-1.

In order to explore the possibility that the low potency of the N-terminal peptides is due to its lack of an independent second binding site, we tested whether activity could be enhanced by co-addition of a folded fragment corresponding to SDF-1, 9-67, which lacks residues 1-8. Thus the entire SDF-1 structure was available to the receptor, but as two separate molecules. SDF-1, 9-67 alone did not bind CXCR4 at the concentrations used (12). Chemotaxis, mobilization of cytosolic free calcium and receptor binding of 1-8 or 1-9 were not affected by the addition of SDF-1, 9-67 (not shown). Thus no synergy could be demonstrated. This does not rule out the possibility that a second site could be involved in full-length SDF-1.

Activity of SDF-1(1-9) dimer. We tested two possible mechanisms that could account for the difference in activity between the 1-9 and 1-8 peptides. The first was whether 1-9 forms a super active dimer. This hypothesis was advanced when we detected dimer in solutions of 1-9. The second was whether the increase in length was responsible. To determine if the extra residue in 1-9, was responsible for its

higher potency we prepared 1-9[Aba-9] a monomer analog of 1-9 with Cys-9 replaced by Aba, a non-thiol-containing amino acid. This peptide had similar activity to the 1-8 peptide (Fig. 2a). To examine the possibility that the 1-9 formed a dimer with higher potency, we oxidized 1-9 and isolated the disulfide bridged dimer. The purified 1-9 dimer had a higher potency than either 1-9 (10-fold); 1-17 (4-fold); 1-8 (75-fold); or the 1-9[Aba-9] monomer analog (36-fold) (Fig 2, Table 1). The activity of 1-9 dimer was still 100-fold less potent than native SDF-1.

To investigate the effect of increasing the peptide length to include both the N-terminal CXC motif and RFFESH binding domains we prepared SDF-1, 1-17. This peptide was more potent than 1-9 but was several fold lower than chemotactic activity than 1-9 dimer (Fig.2a). Dimerization of 1-17 did not affect its potency (not shown).

Receptor binding of the SDF-1 peptides. CEM cells were used to determine the binding of the SDF-1 peptides to CXCR4 (12). The competition for binding of ^{125}I-labeled native SDF-1 by unlabelled native SDF-1 and the N-terminal peptides is shown in Figure 4. The K_d values are summarized in Table 1. The competition by both the 1-8 and 1-9[Aba-9] peptides was incomplete, so a reliable K_d could not be determined. The 1-9 dimer peptide had 82-fold lower affinity than native SDF-1. In comparison the 1-9 peptide had 1500-fold lower affinity than native SDF-1. The affinity of the 1-17 monomer and the 1-9 dimer were very similar (Fig. 4 and Table 1). In contrast to the 1-9, the dimer of 1-17 was not significantly different from the 1-17 monomer (not shown). The K_ds for the 1-9 forms approximately corresponded with the chemotaxis results. To determine whether the 1-9 dimer bind to other chemokine receptors, competition for MCP-1 or RANTES binding to THP-1 cells was measured. THP-1 cells express CXCR4 as well as a number of CC chemokine receptors, including receptors for MCP-1 and RANTES. Like native SDF-1, the peptides did not compete for the binding of either MCP-1 or RANTES (not shown).

An SDF-1, 1-9 analog is an antagonist. A low molecular weight antagonist for SDF-1 could provide a lead for therapeutics. We had previously shown that a full length analog, SDF-1, 1-67[P2G], is a potent

receptor antagonist (12). Therefore we tested the corresponding 1-9 analog, SDF-1, 1-9[P2G], and found that its dimer lacked detectable activity (Fig 2a), but it competed for SDF-1 binding with similar affinity to 1-9 dimer (Fig 4). The 1-9[P2G] dimer inhibited SDF-1 activity in a dose dependent manner (Fig. 3b). 50 μM of 1-9[P2G] dimer was required to inhibit the activity of 10 nM of SDF-1 by 50%, a ratio of 5,000. Therefore, as with full-length SDF-1 (12), this modification converted the 1-9 into an antagonist. Thus we have identified a peptide antagonist of CXCR4.

SDF-1 peptides are specific for CXCR4. Native SDF-1 and the N-terminal peptides induced a rapid and transient rise in cytoplasmic calcium concentration, $[Ca^{2+}]_i$, in T lymphocytes (Fig. 5a) as well as CEM cells (Fig. 6). The rate and magnitude increased with the concentration. Whereas a response to SDF-1 was observed at 1×10^{-9} M, the peptides induced $[Ca^{2+}]_i$ changes in the micromolar range. Receptor usage by the SDF-1 peptides was assessed by monitoring $[Ca^{2+}]_i$ changes after sequential stimulation. As shown in Fig. 5a, treatment of T lymphocytes with SDF-1 completely abolished the responsiveness to the 1-9 peptide, and conversely, the 1-9 peptide also markedly attenuated the response to native SDF-1. The 1-9 dimer (50 μM) completely desensitized the response to subsequent native SDF-1 (not shown). No effect on the response to the 1-9 peptide was observed when T lymphocytes were pre-stimulated with MCP-1, RANTES, MIP-1β, IP10, or Mig (Fig. 5b). The selectivity of these chemokines (1) implies that SDF-1 peptides desensitize CXCR4 but not other chemokine receptors including CXCR3, CCR1, CCR2 and CCR5. No response to eotaxin, I-309 or TARC (Fig. 5b) was obtained with these cells under the conditions used, and as expected, they did not desensitize 1-9.

Specificity of the peptides was further examined using a CXCR4 blocking monoclonal antibody (17). The chemotaxis and calcium induction by SDF-1 and SDF-1, 1-9 dimer were blocked by the antibody (Fig. 6). The response of a control chemokine, secondary lymphoid-tissue chemokine which does not bind CXCR4, was not affected by the antibody (Fig. 6b). Taken together these data show that the 1-9 peptide binds and activates CXCR4, and demonstrate that it is specific for CXCR4.

Discussion.

We have shown that N-terminal SDF-1 peptides bind and activate CXCR4. Peptides corresponding to 1-8, 1-9, 1-9 dimer and 1-17, bind and activate CXCR4, but had low potency compared to SDF-1. Apart from their potency, the agonist peptides were indistinguishable from SDF-1 in all the activities measured. The same substitution, P2G, converted SDF-1 and the 1-9 dimer to a specific SDF-1 antagonist. The binding and activities of the N-terminal peptides were blocked by an antibody directed to CXCR4 confirming that the peptides bind CXCR4.

Although residues in the N-terminal region of chemokines are critical for receptor-activation, N-terminal peptides that have been tested do not bind and stimulate chemokine function. This study demonstrates that SDF-1 is an exception. There are several possible reasons for the difference between SDF-1 and other chemokines. A substantial body of work had lead to a model in which there are two chemokine receptor binding sites, and the initial interaction occurs with a site in the loop region that follows the CXC or CC motif (12-15, 19-21). This is proposed to facilitate the subsequent binding of the N-terminal region to a buried site in the receptor (12, 15). Whereas interaction of a structured loop region with the receptor could be required for the activity of most chemokine N-terminal peptides (15), our data indicates this not essential for the SDF-1 peptides. Alternatively, the lack of binding of the N-terminal peptides of other chemokines is due to the failure to adopt a receptor bound conformation. The ^{1}H-NMR structure of SDF-1 shows that the N-terminal region is entirely solvent accessible and has no detectable secondary structure and therefore is expected to be highly flexible. However it is reasonable to propose that the N-terminal region adopts a well defined conformation when SDF-1 binds to CXCR4. Similarly for the N-terminal peptide the receptor bound conformation could be represented in solution. Nevertheless a prerequisite for binding of the N-terminal peptide to CXCR4 is that the receptor binding site must be accessible. For other chemokines there is no evidence that this site is accessible. One possibility is that for binding of the N-terminal domain, other chemokine-receptor interactions are first required. This exposes the receptor site and allows the N-terminal domain to bind. In this model N-terminal peptides would not bind. In CXCR4 the loops of the receptor could be arranged such that this binding site is

accessible to the peptide, whereas for most chemokines even if the N-terminal peptide existed in the bound conformation the pathway to the receptor could be sterically blocked without the remainder of the chemokine. Thus the difference between SDF-1 and other chemokines might not be in the peptide ligand, but in the receptor.

The affinity and potency of the N-terminal SDF-1 peptides are lower than those of the native protein. Thus the peptides can bind CXCR4, but not as efficiently as native SDF-1. Similar arguments to those above could account for the difference in potency. The low affinity could be due to the lack of the loop binding site on the N-terminal peptide or to multiple conformations of the peptide and or a requirement for binding cooperativity with other regions of the protein.

The disulfide-linked dimer of SDF-1, 1-9 peptide was considerably more potent than the 1-9 peptide monomer. Although 1-9 was isolated as the monomer it had higher activity than an analog that could not dimerize suggesting that the 1-9 spontaneously starts to form dimer in solution. In native SDF-1, Cys-9 normally participates in a disulfide bridge. Thus, the disulfide bridge itself could directly enhance binding to the receptor. A second alternative is that dimerization could change the conformation of the 1-9 resulting in an enhanced binding. The fact that native SDF-1 binds as a monomer, and not as a dimer (12), indicates that only one 1-9 can binds to the receptor site. A third possibility is that one half of the dimer binds the activation site, but the other half could bind to other receptor sites, perhaps due to fortuitous complementarity. This last mechanism is consistent with the finding that the 1-17 has similar binding to the 1-9 dimer. The 1-17 contains the RFFESH motif, which is a receptor binding site on SDF-1. Further experiments will be required to determine the detailed mechanisms involved.

Stable low molecular weight non-peptide ligands are preferred for therapeutic applications. SDF-1 is the co-receptor for HIV and is involved in hemopoietic cell homing. Several non-chemokine molecules have been found to inhibit HIV and it was shown that they target CXCR4 (22-24). However none of these have SDF-1 activity. Antagonists of chemokines are likely to be the most useful variants for therapeutic usage, and we have demonstrated that modification to N-terminal sites of several chemokines, including

SDF-1, results in antagonists. SDF-1, 1-9[P2G] dimer is the first peptide chemokine antagonist. Many 7-transmembrane receptors have small molecule natural ligands and have been successfully targeted by analogs to generate pharmaceutical compounds. However protein ligands such as chemokines, which have larger binding surfaces and depend on cooperative interactions present a more complex chemical problem. The results with SDF-1 peptide antagonists indicate that it would be feasible to target the N-terminal region. These peptides could be leads for the generation of low molecular weight high affinity CXCR4 agonists or antagonist.

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Footnotes

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1 Abbreviations used:

SDF-1, stromal cell derived factor-1; Aba, α-amino-(n)-butyric acid; CXCR, CXC chemokine receptor; CCR, CC chemokine receptor; $[Ca^{2+}]_i$ intracellular concentration of calcium ions; GROα, growth related protein-α; IL-8 interleukin-8; IP10, γ-interferon inducible protein-10; Mig, monokine induced by interferon-γ; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation normal T cell expressed; TARC, thymus and activation-regulated chemokine.

Table 1. Summary of the relative potencies of SDF-1 peptides

	Binding		Chemotactic activity	
Peptides	K_ds (nM)[a]	Fold increase[b]	EC50 (nM)[c]	Fold increase[d]
SDF-1	9 ± 3	1	5 ± 1	1
SDF, 1-8	e		37,500 ± 10,600	7,500
SDF, 1-9	13,900 ± 5,500	1,500	5,200 ± 3,800	1,040
SDF, 1-9[Aba-9]	e		17,800	3,600
SDF, 1-9 Dimer	730 ± 90	82	500	100
SDF, 1-9[P2G] Dimer	2,580	290	f	–
SDF, 1-17	850 ± 26	94	2,200 ± 490	440

[a] K_ds were calculated from CEM cell binding curves derived in 2-6 experiments, with results similar to those in Fig. 4, using Scatchard methods.

[b] The fold increase in K_d was calculated relative to native SDF-1.

[c] The chemotaxis EC50 was calculated from the CEM cell data in Fig. 2*a*. Results are presented as the mean ± SD from 2 experiments.

[d] Fold increase in chemotaxis EC50 was calculated relative to native SDF-1.

[e] K_d not determined (see text).

[f] not detectable

Figure legends

Figure 1. Sequences of native SDF-1 and the N-terminal SDF-1 peptides.

Figure 2. Chemoattractant activity of SDF-1 peptides. *(a)* Migration of CEM cells in response to the SDF-1 peptides: 1-8 (❑); 1-9 (Δ); 1-17 (×); 1-9 dimer (▲); and 1-9[Aba] (⊞); 1-9[P2G] dimer (■), and native SDF-1 (●). *(b)* Migration of T-lymphocytes: 1-8 (❑); 1-9 (Δ); 1-9 dimer (▲) native SDF-1 (●). Data are shown is the mean ± SD of migrated cells. Similar results were obtained in two additional experiments.

Figure 3. Chemotaxis inhibition by SDF-1 and SDF-1, 1-9 antagonists. *(a)* CEM cell migration induced by SDF-1, 1-9 peptide (10 μM) in the presence of the indicated concentrations of the antagonist, SDF-1, 1-67[P2G] (⊡); or the IL-8 antagonist, IL-8, 6-72 (⊙). Migration is expressed as percent of the response obtained in the absence of antagonist (control, ■). *(b)* Antagonist activity of the SDF-1, 1-9[P2G] dimer. SDF-1 agonist was added to the bottom well at a concentration of 10 nM and SDF-1, 1-9[P2G] dimer added at the indicated concentrations (■). The percent migration of SDF-1 alone (●) is shown. Data are the mean ± SD of duplicate determinations from 2 separate experiments.

Figure 4. Receptor binding of SDF-1 peptides. Competition for specific binding of ^{125}I-SDF-1 (4 nM) to CEM cells by 1-8 (❑); 1-9 (Δ); 1-17 (×); 1-9 dimer (▲); 1-9[Aba-9] (⊞); 1-9[P2G] dimer (■); and native SDF-1 (●). The percentage specific cpm bound in the absence of competitor (⊠), is shown. The results are representative from 2 to 6 experiments.

Figure 5. Receptor selectivity of the SDF-1 peptides. T lymphocytes that were loaded with Fura-2 were sequentially stimulated with chemokines and SDF-1, 1-9 and the resulting $[Ca^{2+}]_i$ dependent fluorescence changes were recorded. *(a)* Cross-desensitization of SDF-1 and the 1-9 peptide. *(b)*

Lack of desensitization of SDF-1, 1-9 by the indicated CXC or CC chemokines. The chemokines were added at 100 nM, except for SDF-1 which was added at 1 nM, followed by addition of the 1-9 peptide (30 μM) after 60 s. The results shown are representative of 2-3 independent experiments.

Figure 6. CXCR4 selectivity of the SDF-1, 1-9 dimer. *(a)* Chemotaxis of CEM cells in response to SDF-1 (5 nM) or SDF-1, 1-9 dimer (1 μM) was measured in the presence or absence of anti CXCR4 monoclonal antibody 12G5 (10 $\mu g.ml^{-1}$). *(b)* CEM cells that were either pretreated with antibody or untreated were loaded with Fura–2 and stimulated with SDF-1 (3 nM) or SDF-1, 1-9 dimer (10 μM), followed by 100 nM secondary lymphoid-tissue chemokine (SLC) after 60 s. The Ca^{2+} -dependent changes in fluorescence were recorded.

We claim:

1. The use of CXCR4 antagonists for treating cancer.

2. CXCR4 antagonists comprising stromal cell derived factor-1 wherein glycine is substituted for proline at position 2.

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                  1           11          21          31
                  |           |           |           |
SDF-1             KPVSLSYRCP  CRFFESHVAR  ANVKHLKILN  TPNCALQIVA

                              41          51          61
                              |           |           |
                              RLKNNNRQVC  IDPKLKWIQE  YLEKALN
```

SDF-1, 1-8 KPVSLSYR

SDF-1, 1-9 KPVSLSYRC

SDF-1, 1-9 [Aba] KPVSLSYRAba

SDF-1, 1-9 dimer

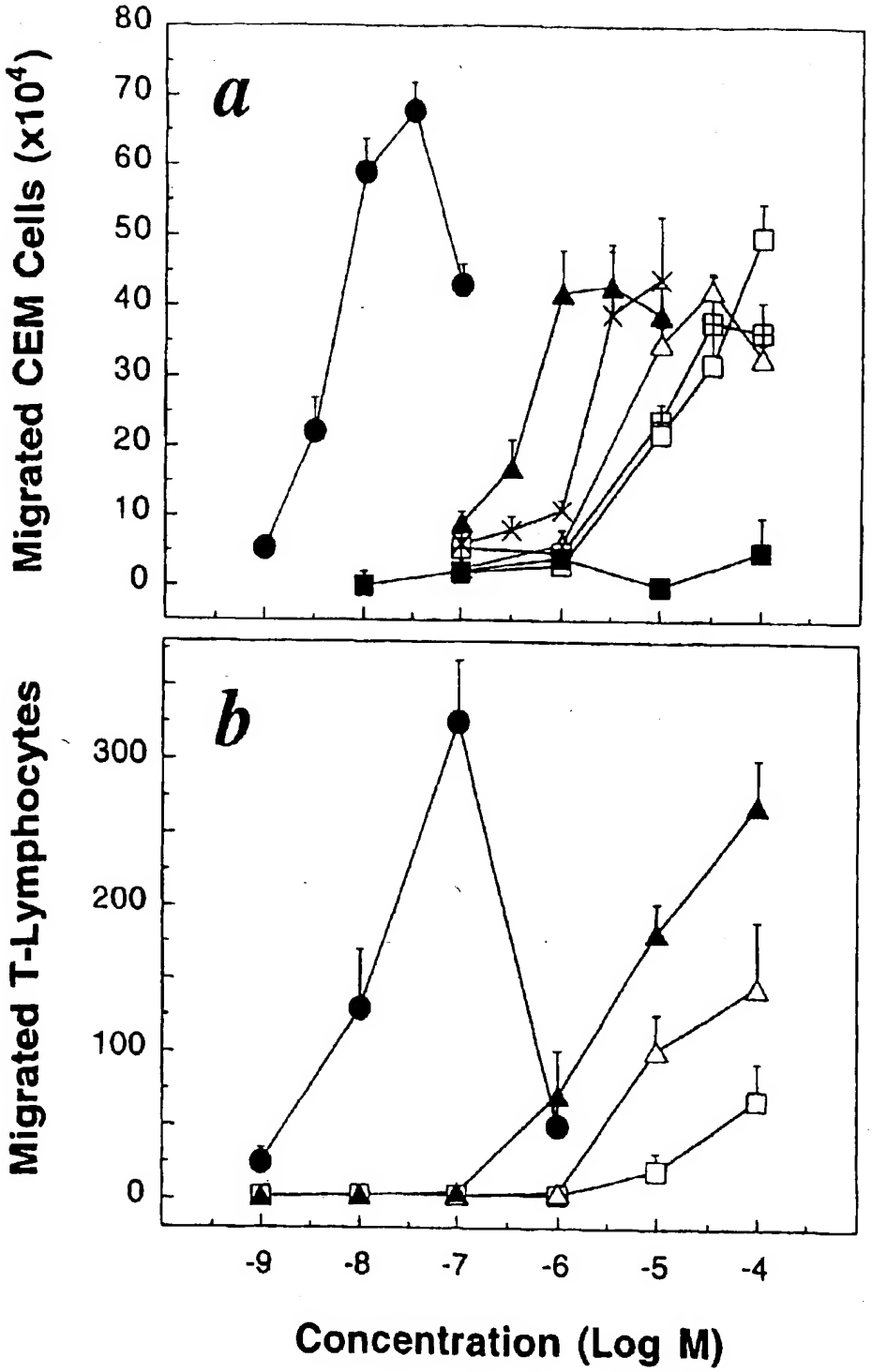
```
KPVSLSYRC
         |
KPVSLSYRC
```

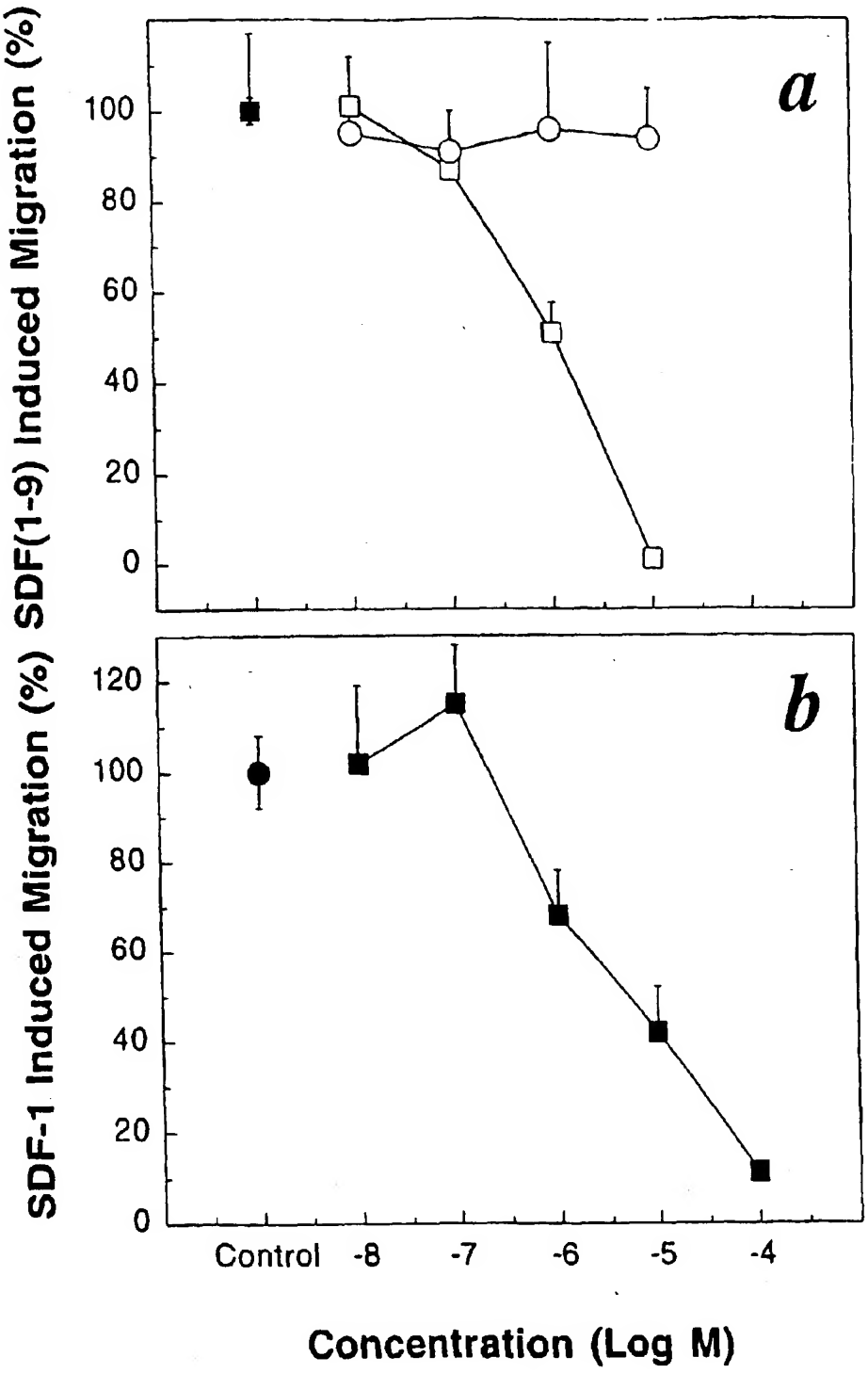
SDF-1, 1-9[P2G] dimer

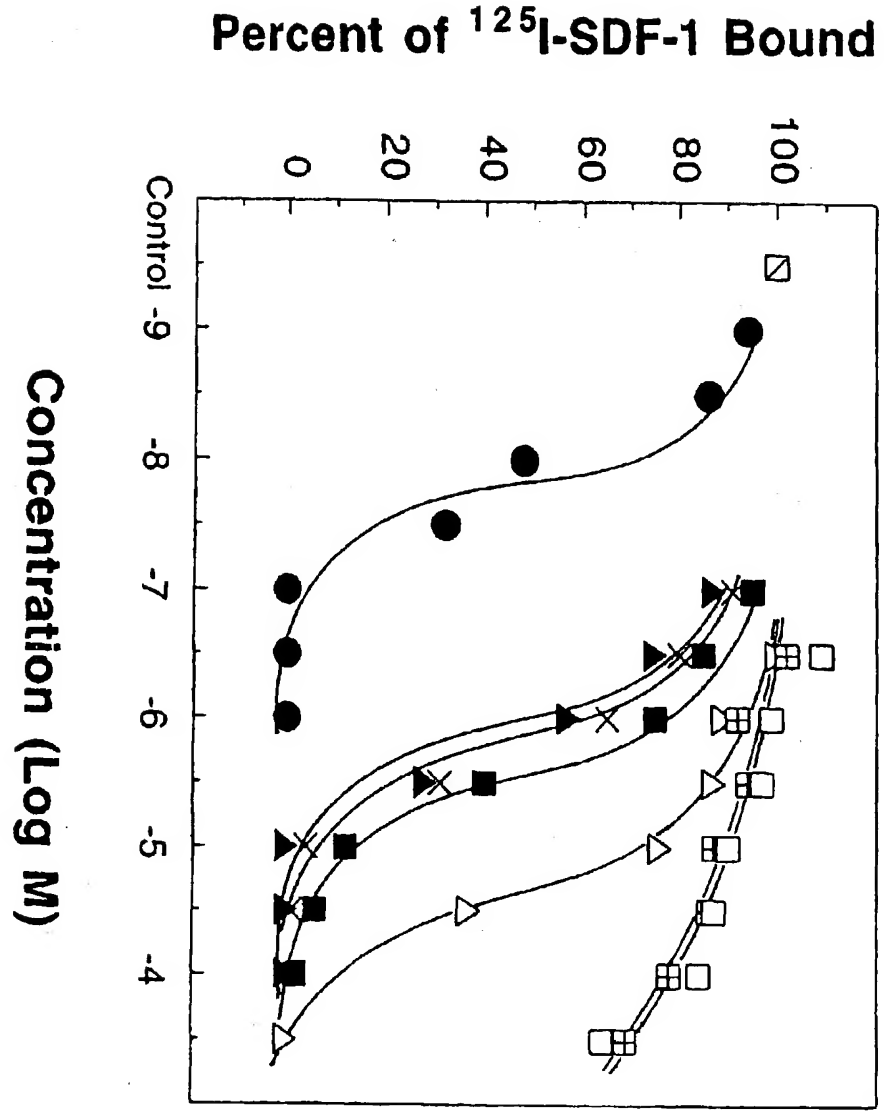
```
KGVSLSYRC
         |
KGVSLSYRC
```

SDF-1, 1-17 KPVSLSYRCPCRFFESH

Fig. 1


a
Migrated CEM Cells (x10⁴)
80
70
60
50
40
30
20
10
0
b
Migrated T-Lymphocytes
300
200
100
0
-9
-8
-7
-6
-5
-4
Concentration (Log M)


a
b
100
80
60
40
20
0
120
100
80
60
40
20
0
Control
-8
-7
-6
-5
-4
SDF-1 Induced Migration (%) SDF(1-9) Induced Migration (%)
Concentration (Log M)


Percent of ^{125}I-SDF-1 Bound
0
20
40
60
80
100
Control
-9
-8
-7
-6
-5
-4
Concentration (Log M)

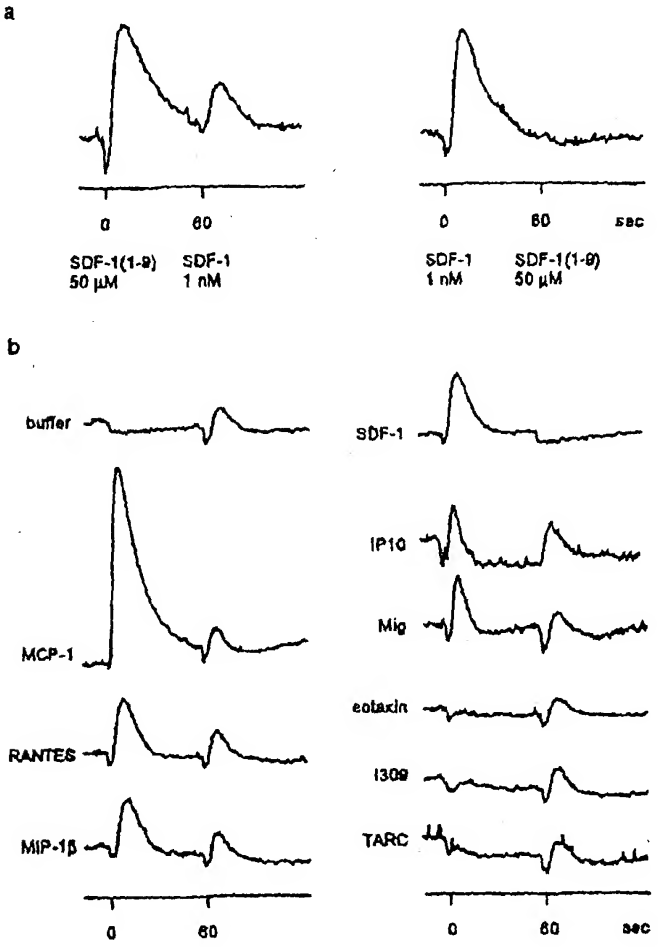
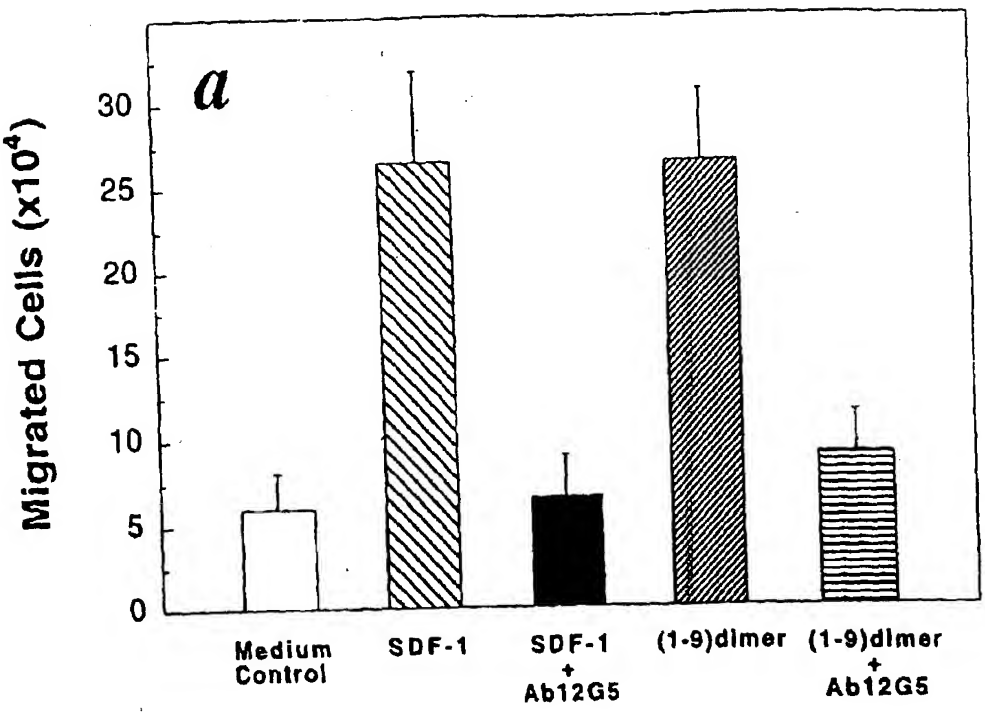
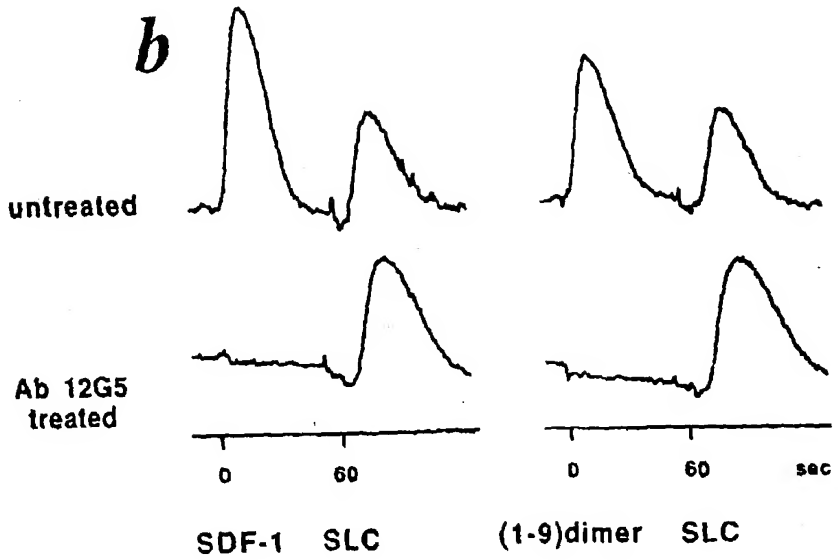

a
0
60
SDF-1(1-9)
50 µM
SDF-1
1 nM
0
60
sec
SDF-1
1 nM
SDF-1(1-9)
50 µM
b
buffer
SDF-1
IP10
MCP-1
Mig
eotaxin
RANTES
I309
MIP-1β
TARC
0
60
0
60
sec

Fig. 5


a
Migrated Cells (x10⁴)
30
25
20
15
10
5
0
Medium Control
SDF-1
SDF-1 + Ab12G5
(1-9)dimer
(1-9)dimer + Ab12G5


b
untreated
Ab 12G5 treated
0
60
0
60
sec
SDF-1 SLC
(1-9)dimer SLC